Chapter VII

Discussion

Inflammation

It is generally assumed that tissue injury leads to local inflammation, and if the extent is large enough, tissue injury also leads to systemic inflammation. Such a “classical local inflammatory response” as reflected by an immediate granulocyte migration into the damaged tissue may result from a variety of stimuli like ischemia reperfusion injury (1-4), skin injury (5;6), infection (7) and mucosal damage (8;9). Inflammation can be restricted to the site of tissue damage but is often accompanied to some extent by a systemic response (10). In general, inflammation is a strictly controlled system: duration and intensity of the inflammatory response is carefully regulated in order to limit tissue damage as a result of a too extensive reaction.

When the inflammatory response is large enough to cause systemic effects, like defined (11-13) changes in temperature, heart rate, respiratory rate or white blood cell count, we call it the systemic inflammatory response syndrome (SIRS). In case of persistent activation, the systemic inflammatory response can result in a type of deleterious unregulated inflammation known as multiple organ dysfunction syndrome (MODS).Unregulated inflammation as can be observed in multi-trauma patients, burn patients or patients with otherwise devitalized tissues (abdominal abcesses, pancreatitis), is characterized by an excessive release of pro-inflammatory mediators such as cytokines, acute phase proteins, soluble adhesion molecules and complement products (14-17).

Can a local trigger lead to systemic inflammation?

Although many data concerning the presence of circulating markers of inflammation in relation to an unregulated systemic inflammation are available in multi-trauma patients, underlying mechanisms are largely unknown. An obvious explanation is, that the systemic response is induced by the local inflammatory response. In the systemic circulation measured cytokines are quantitatively related to the degree of tissue damage (18-21). If there is an ongoing local activation in the damaged tissue with a spill-over of locally produced inflammatory mediators into the systemic circulation, an unregulated systemic response may be induced.

The importance of a local inflammatory response in initiating an unregulated systemic response is underscored by the finding that early fixation of long bone fractures increases survival of multi-trauma patients (22-26). Immediate stabilization of long bone fractures prevents ongoing movement at the fracture site. By this, repetitive local damage to the surrounding tissue is prevented.

There are similarities in the described systemic inflammatory response to trauma as indicated above and in the fat embolism syndrome. Marrow fat embolization occurs in almost all patients with long bone or pelvic fractures

(27;28). Only a minority of these patients develop mental disturbances, pulmonary stress, petechiae and early fever, all symptoms related to the classical fat embolism syndrome. The fat embolism syndrome develops more often in patients with a closed fracture and, or delayed (i.e. after 24 hours) surgery than in patients with an open fracture or early operation (27;29). This implies that in addition to purely mechanical factors (embolization of fat) other factors possibly play a role in the development of systemic signs of fat embolism. Accompanying soft tissue injury surrounding the long bone fracture has been proposed to be such an additional factor (30).

Since local inflammation seems important in response to injury and in initiating the systemic response, it was studied whether indicators of an acute local inflammatory response (within 12 hours post-injury) could be found in locally inflicted tissue in the case of blunt trauma. More specifically, local inflammation was studied in the light of the assumption that tissue damage in tissues surrounding a fractured bone is followed by a local inflammatory response.

Local inflammation in skin after blunt trauma

Local inflammation as a result of blunt trauma has first been studied in human skin. Skin is the largest “organ” of the body and represents the interface between the internal and external environment. It is a “barrier” and therefore an important protective organ. In addition, skin contains many immunocompetent cells such as Langerhans cells, keratinocytes, fibroblasts, mast cells, tissue macrophages and lymphocytes. It is generally assumed that skin plays an active role in inducing a local inflammatory response (31-33).

To assess this in trauma patients, we have compared skin surrounding a closed femoral fracture with remote (in the same patient) and normal skin and biopsies were analyzed using immunohistochemistry, electron microscopy, ELISA and RT-PCR. To our surprise, no signs of a local inflammatory response were observed in skin after blunt trauma. Using immunohistochemistry, no infiltrating granulocytes were observed and there was no increase in pro-inflammatory cytokine expression. Skin surrounding the fracture, but also remote skin showed limited activation as was apparent from low or absent E-selectin expression in local endothelial cells, generally low IL1β and IL6 mRNA expression and low TNFα and IL6 protein expression. Electron microscopy showed that there is a wide variation in the morphology of endothelial cells indicative for variations in activation status of endothelial cells. However also by this method no clear signs of an inflammatory response were observed. Altogether, these data indicate that local inflammation as a result of blunt trauma is limited in skin. Apparently, the mechanical impact, necessary to fracture the

underlying bone does not result in damage of the skin and a subsequent local inflammatory response.

Local inflammation in muscle after blunt trauma

Since skeletal muscle is located more closely to a fractured bone than skin, it might become damaged more easily. From clinical experience we know that muscle often becomes edematous after long bone fractures. As with edema in other tissues, muscle swelling is considered to be part of an inflammatory response. In case a long bone fracture is attended by serious local muscle swelling, it is often necessary to perform a fasciotomy to prevent a compartment syndrome (high pressure soft tissue ischemia) (34;35).

Although in our study muscle was found to be damaged and signs of bleeding were present between muscle fibers as a result of blunt trauma, no signs of a local inflammatory response were observed. No infiltrating cells, no induction of adhesion molecule expression nor induction of pro-inflammatory cytokines were found in muscle biopsies with immunohistochemistry. RT-PCR experiments confirmed that there is no substantial increase in cytokine production as a result of blunt trauma.

Since swelling of the muscle reaches maximum values after 24 to 48 hours after the initial injury, signs of local inflammation may be observed at later time points. But, based on the knowledge that granulocytes quickly enter damaged tissue, it is unlikely that these cells will be found later. Monocytes and macrophages might be observed after one or two days. However, it remains unclear if there is a direct relation between these cell types and the observed clinical picture. So, in conclusion, blunt trauma does not induce a “classical local inflammatory response” in muscle.

Local inflammation in adipose tissue after trauma

In addition to skin and muscle, subcutaneous adipose tissue was studied for the early production of pro-inflammatory cytokines as a result of blunt trauma. Blunt trauma induced local activation in adipose tissue which was demonstrated by a clear increase in IL6 and IL8 in adipose tissue near the fracture as compared to remote adipose tissue (from the same patient, but taken from the hip region). RT-PCR experiments, showing the presence of TNFα, IL6 and IL8 mRNA in adipose tissue, indicate that the increased cytokine levels are the result of actual production by adipose tissue itself. The observed pro-inflammatory cytokines can originate from adipocytes, but it can not be excluded that these are produced by other cells present in adipose tissue, such as endothelial cells and fibroblasts. Since granulocytes are not present in skin and muscle surrounding the fractured bone, it is unlikely to expect infiltrating

granulocytes in subcutaneous adipose tissue which is located between skin and muscle.

Thus although adipose tissue is clearly activated as opposed to skin and muscle, a “classical local inflammatory response” with granulocyte infiltrates is probably not induced.

Blunt trauma does not induce a “classical local inflammatory response”

Starting from the principle that every injury should lead to an inflammatory response, we assumed that blunt trauma (a closed femoral fracture) would induce a “classical local inflammatory response” as characterized by infiltrating granulocytes. In contrast to what we expected, no such reaction was observed in skin and muscle surrounding the fractured bone. Although there is a possibility that an inflammatory response occurs later, a granulocyte infiltrate more than 12 hours post injury would not be expected. Remarkable is the fact that we did observe “inflammation” in adipose tissue as a result of a femoral fracture. Yet, this does not mean that a “classical inflammatory response” with infiltrating cells was induced.

Since in contrast to skin and muscle, there is cytokine production in adipose tissue, different activation signals and subsequent mechanisms are induced in adipose tissue than in skin and muscle. So, the type of tissue that is involved seems to be an important factor in the subsequent response.

Local inflammation as a result of different injuries

Since no “classical inflammatory response” was observed in tissues after blunt trauma, other kinds of injuries were studied: a simple skin incision and the late effects (> 48 hours) of penetrating injuries, i.e. one which leads to infection, of the skin.

In contrast to blunt trauma, the other types of injury did indeed induce local inflammation that was accompanied by granulocyte infiltration.

First, local inflammation after disruption of the epithelial skin barrier was studied. Surgical incision under aseptic conditions was used as a model. We observed granulocyte infiltration at first at 30 minutes after incision of the skin, their number increasing with time. This granulocyte infiltration was paralleled by E-selectin expression on endothelial cells, whereas no P-selectin, ICAM-1 and VCAM-1 expression was induced. Comparable to what was observed after blunt trauma, incision of the skin did not result in observable changes in pro-inflammatory cytokines (TNFα, IL1α, IL1β, IL6, IL8). This is probably the result of the limited extent of tissue damage and the relative short study period (up to 4.5 hours) (6).

Second, local inflammation as the late result of a penetrating injury (splinter, bite, infectious arterial line) resulting in infection was studied. This time a more pronounced and different inflammatory pattern was observed. Not only granulocytes, but also a clear increase in monocyte /macrophages and T-cells was found. Furthermore, E-selectin, VCAM-1 and increased ICAM-1 is observed. In contrast to skin afflicted with a simple incision, infected skin showed a markedly changed cytokine pattern. TNFα and IL1α expression is increased, whereas IL8 expression was decreased. Although the different time points at which incised and infected skin were studied can explain the different responses, the magnitude and seriousness of the response is probably a result of the type of injury (36): sharp wound edges without a lot of clear tissue damage versus contused wound edges with much apparently non-vital tissue. These latter forming an ideal environment for bacterial outgrowth.

Different local inflammatory responses in skin, muscle and adipose tissue

Skin

Based on the findings that a penetrating injury and even aseptic incision of the skin does induce a “classical inflammatory response”, whereas blunt trauma does not, we propose that disruption of the epithelial barrier is an important trigger for granulocyte infiltration in human skin. This is confirmed by the fact that an open fracture induces a local inflammatory response similar as is observed in incised skin, namely recruitment of granulocytes in combination with E-selectin expression (unpublished results). Similar responses as seen in skin are also observed in the mucosal barrier of the gastrointestinal tract. In ulcer formation, the initial trigger for inflammation is a broken mucosal epithelial barrier as a result of injury, chemicals, drugs or toxins (8;9;37). Apparently, disruption of the epithelial barrier in general is an important trigger for the induction of local inflammation.

In addition to a disrupted epithelial barrier, the presence of noxious substances is also a trigger for the induction of a “classical local inflammatory response”. This is in agreement with reports about inflammation in human skin as a result of injury: burns, disruption of the skin, tape stripping, and intra-dermal injection or applying of irritant substances (7;38-42). These are all stimuli that directly activate or damage cells in the skin or interfere with the epithelial skin barrier. Our findings concerning the local response as a result of infection after a penetrating injury also indicated that the presence of dead tissue and subsequent grow of bacteria had clearly pro-inflammatory effects in addition to the effects observed after disruption of the epithelial barrier under aseptic conditions (simple skin incision).

From the point of view that skin provides protection and epithelial cells in general serve as a barrier, it is comprehensible that an inflammatory response with a granulocyte infiltrate is induced after incision of the skin or after an open fracture: when the epithelial barrier is broken, there is a clearly increased risk for infection. In the case of a closed femoral fracture, the presence of only damage from inside without disruption of the epithelial barrier and the low risk for infection might explain the absence of a “classical local inflammatory response”.

Muscle

The fact that muscle is swollen as a result of blunt trauma apparently does not mean that there is a “classical” local response with subsequent granulocyte infiltrate, as we demonstrated. As muscle is damaged from inside, granulocytes are not necessary, because similar as in skin, there is no risk for infection. At later time points, macrophages may be present in muscle to contribute to the repair of the damaged tissue. However they play as far as we know no distinct role in the induction of the acute inflammatory response characterized by capillary leakage and soft tissue swelling. Since not any sign of an inflammatory response was observed in the studied muscle biopsies, the underlying pathophysiological process remains obscure.

Helliwell et al. (43) observed endothelial activation in muscle of patients with multiple organ failure. The biopsies they studied were taken after at least one day on the intensive care unit and thus also at least one day after the initial injury. They conclude that endothelial activation in muscle as a primary process and not microvascular damage and ischemia may be an important factor in pathogenesis of multiple organ failure. This observation points to a systemic response rather than a local response (like the swelling of a muscle compartment in case of a broken leg) and is of no help in the interpretation of our results.

Adipose tissue

Blunt trauma clearly induces IL6 and IL8 production in adipose tissue in the early phase (within 12 hours) after blunt trauma. This indicates that in contrast to skin and muscle, blunt trauma has a direct effect on adipose tissue. Pro-inflammatory cytokines derived from adipose tissue may constitute the link between local and systemic inflammation while it is known that adipose tissue derived IL6 can be released into the circulation (44;45). Also, a positive association has been reported between body mass index (BMI) and elevated CRP levels. CRP is one of the inflammatory parameters, which production is induced by IL6. So, elevated CRP can be explained by increased IL6 production in patients with obesity or overweight (46). These data indicate that pro-inflammatory cytokines produced by adipose tissue can contribute to a systemic

inflammatory response (47;48). The same cytokines may also induce endothelial cell activation in tissues surrounding the fracture, such as muscle.

Concluding remarks

We did not observe an early “classical local inflammatory response” in our model of blunt trauma - a closed femoral fracture. Since tissues surrounding the fractured bone do not come in contact with the “foreign” environment, there is no increased risk for infection. As a result of this, there is no need for granulocyte infiltration.

It is concluded that the mechanical injury as a result of a femoral fracture only induces cytokine production in adipose tissue and not in skin and muscle tissue within the observation period. So, inflammation of adipose tissue may be the main cause of elevated IL6 levels observed early after blunt trauma.

References

1. Seekamp, A., Till, G. O., Mulligan, M. S., Paulson, J. C., Anderson, D. C., Miyasaka, M., and Ward, P. A. Role of Selectins in Local and Remote Tissue Injury Following Ischemia and Reperfusion. Am.J.Pathol. 1994;144(3):592-8.

2. Verrier, E. The Microvascular Cell and Ischemia-Reperfusion Injury. J.Cardiovasc.Pharmacol. 1996;27 Suppl 1:S26-S30.

3. Groeneveld, A. B., Raijmakers, P. G., Rauwerda, J. A., and Hack, C. E. The Inflammatory Response to Vascular Surgery-Associated Ischaemia and Reperfusion in Man: Effect on Postoperative Pulmonary Function. Eur.J.Vasc.Endovasc.Surg. 1997;14(5):351-9.

4. Sullivan, G. W., Sarembock, I. J., and Linden, J. The Role of Inflammation in Vascular Diseases. J.Leukoc.Biol. 2000;67(5):591-602.

5. Singer, A. J. and Clark, R. A. Cutaneous Wound Healing. N.Engl.J.Med. 2-9-1999;341(10):738-46.

6. van der, Laan N., de Leij, L., and ten Duis, H. J. Local Cellular Inflammation As a Result of Elective Standardized Vascular Surgery. Acta Histochem. 2001;103(2):139-49.

7. Silber, A., Newman, W., Reimann, K. A., Hendricks, E., Walsh, D., and Ringler, D. J. Kinetic Expression of Endothelial Adhesion Molecules and Relationship to Leukocyte Recruitment in Two Cutaneous Models of Inflammation. Lab.Invest. 1994;70(2):163-75.

8. Ernst, P. B., Crowe, S. E., and Reyes, V. E. How Does Helicobacter Pylori Cause Mucosal Damage? The Inflammatory Response. Gastroenterology 1997;113(6 Suppl):S35-S42.

9. Podolsky, D. K. Mucosal Immunity and Inflammation. V. Innate Mechanisms of Mucosal Defense and Repair: the Best Offense Is a Good Defense. Am.J.Physiol 1999;277(3 Pt 1):G495-G499.

10. Gabay, C. and Kushner, I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. N.Engl.J.Med. 11-2-1999;340(6):448-54.

11. Beal, A. L. and Cerra, F. B. Multiple Organ Failure Syndrome in the 1990s. Systemic Inflammatory Response and Organ Dysfunction. JAMA 19-1-1994;271(3):226-33.

12. Davies, M. G. and Hagen, P. O. Systemic Inflammatory Response Syndrome. Br.J.Surg. 1997;84(7):920-35.

13. Brun-Buisson, C. The Epidemiology of the Systemic Inflammatory Response. Intensive Care Med. 2000;26 Suppl 1:S64-S74.

14. Goris, R. J., te-Boekhorst, T. P., Nuytinck, J. K., and Gimbrere, J. S. Multiple-Organ Failure. Generalized Autodestructive Inflammation? Arch.Surg. 1985;120(10):1109-15.

15. Goris, R. J. MODS/SIRS: Result of an Overwhelming Inflammatory Response? World J.Surg. 1996;20(4):418-21.

16. Yao, Y. M., Redl, H., Bahrami, S., and Schlag, G. The Inflammatory Basis of Trauma/Shock-Associated Multiple Organ Failure. Inflamm.Res. 1998;47(5):201-10.

17. Kim, P. K. and Deutschman, C. S. Inflammatory Responses and Mediators. Surg.Clin.North Am. 2000;80(3):885-94.

18. Bitterman, H., Kinarty, A., Lazarovich, H., and Lahat, N. Acute Release of Cytokines Is Proportional to Tissue Injury Induced by Surgical Trauma and Shock in Rats. J.Clin.Immunol. 1991;11(4):184-92.

19. Baigrie, R. J., Lamont, P. M., Kwiatkowski, D., Dallman, M. J., and Morris, P. J. Systemic Cytokine Response After Major Surgery. Br.J.Surg. 1992;79(8):757-60.

20. Moore, F. A. and Moore, E. E. Evolving Concepts in the Pathogenesis of Postinjury Multiple Organ Failure. Surg.Clin.North Am. 1995;75(2):257-77.

21. Gebhard, F., Pfetsch, H., Steinbach, G., Strecker, W., Kinzl, L., and Bruckner, U. B. Is Interleukin 6 an Early Marker of Injury Severity Following Major Trauma in Humans? Arch.Surg. 2000;135(3):291-5.

22. Goris, R. J., Gimbrere, J. S., van Niekerk, J. L., Schoots, F. J., and Booy, L. H. Early Osteosynthesis and Prophylactic Mechanical Ventilation in the Multitrauma Patient. J.Trauma 1982;22(11):895-903.

23. Bone, L. B., Johnson, K. D., Weigelt, J., and Scheinberg, R. Early Versus Delayed Stabilization of Femoral Fractures. A Prospective Randomized Study. J.Bone Joint Surg.Am. 1989;71(3):336-40.

24. Pape, H. C., Auf'm'Kolk, M., Paffrath, T., Regel, G., Sturm, J. A., and Tscherne, H. Primary Intramedullary Femur Fixation in Multiple Trauma Patients With Associated Lung Contusion--a Cause of Posttraumatic ARDS? J.Trauma 1993;34(4):540-7.

25. Livingston, D. H. Management of the Surgical Patient With Multiple System Organ Failure. Am.J.Surg. 1993;165(2A Suppl):8S-13S.

26. Livingston, D. H. and Deitch, E. A. Multiple Organ Failure: a Common Problem in Surgical Intensive Care Unit Patients. Ann.Med. 1995;27(1):13-20.

27. ten-Duis, H. J., Nijsten, M. W., Klasen, H. J., and Binnendijk, B. Fat Embolism in Patients With an Isolated Fracture of the Femoral Shaft. J.Trauma. 1988;28(3):383-90.

28. van den Brand, J. G., van der Hoeven, J. H., and Olsman, J. G. [Dyspnea or Confusion After Trauma? Consider Fat Embolism Syndrome]. Ned.Tijdschr.Geneeskd. 5-8-2000;144(32):1513-7.

29. Riska, E. B. and Myllynen, P. Fat Embolism in Patients With Multiple Injuries. J.Trauma 1982;22(11):891-4.

30. ten-Duis, H. J. The Fat Embolism Syndrome. Injury. 1997;28(2):77-85.

31. Kupper, T. S. Immune and Inflammatory Processes in Cutaneous Tissues. Mechanisms and Speculations. J.Clin.Invest. 1990;86(6):1783-9.

32. Bos, J. D. and Kapsenberg, M. L. The Skin Immune System: Progress in Cutaneous Biology. Immunol.Today 1993;14(2):75-8.

33. Bos, J. D. The Skin As an Organ of Immunity. Clin.Exp.Immunol. 1997;107 Suppl 1:3-5.

34. Tarlow, S. D., Achterman, C. A., Hayhurst, J., and Ovadia, D. N. Acute Compartment Syndrome in the Thigh Complicating Fracture of the Femur. A Report of Three Cases. J.Bone Joint Surg.Am. 1986;68(9):1439-43.

35. Heckman, M. M., Whitesides, T. E., Jr., Grewe, S. R., and Rooks, M. D. Compartment Pressure in Association With Closed Tibial Fractures. The Relationship Between Tissue Pressure, Compartment, and the Distance From the Site of the Fracture. J.Bone Joint Surg.Am. 1994;76(9):1285-92.

36. van der, Laan N., de Leij, L., and ten-Duis, H. J. Immunohistopathological Appearance of Three Different Types of Injury in Human Skin. Inflamm.Res. 2001;50(7).

37. Blecker, U. and Gold, B. D. Gastritis and Peptic Ulcer Disease in Childhood. Eur.J.Pediatr. 1999;158(7):541-6.

38. Enk, A. H. and Katz, S. I. Early Events in the Induction Phase of Contact Sensitivity. J.Invest.Dermatol. 1992;99(5):39S-41S.

39. Garner, W. L., Rodriguez, J. L., Miller, C. G., Till, G. O., Rees, R. S., Smith, D. J., and Remick, D. G. Acute Skin Injury Releases Neutrophil Chemoattractants. Surgery 1994;116(1):42-8.

40. Boehm, K. D., Yun, J. K., Strohl, K. P., Trefzer, U., Haffner, A., and Elmets, C. A. In Situ Changes in the Relative Abundance of Human Epidermal Cytokine Messenger RNA Levels Following Exposure to the Poison Ivy/Oak Contact Allergen Urushiol. Exp.Dermatol. 1996;5(3):150-60.

41. Engelhardt, E., Toksoy, A., Goebeler, M., Debus, S., Brocker, E. B., and Gillitzer, R. Chemokines IL-8, GROalpha, MCP-1, IP-10, and Mig Are Sequentially and Differentially Expressed During Phase-Specific Infiltration of Leukocyte Subsets in Human Wound Healing. Am.J.Pathol. 1998;153(6):1849-60.

42. Reilly, D. M. and Green, M. R. Eicosanoid and Cytokine Levels in Acute Skin Irritation in Response to Tape Stripping and Capsaicin. Acta Derm.Venereol. 1999;79(3):187-90.

43. Helliwell, T. R., Wilkinson, A., Griffiths, R. D., Palmer, T. E., McClelland, P., and Bone, J. M. Microvascular Endothelial Activation in the Skeletal Muscles of Patients With Multiple Organ Failure. J.Neurol.Sci. 21-1-1998;154(1):26-34.

44. Mohamed, Ali, V, Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., Klein, S., and Coppack, S. W. Subcutaneous Adipose Tissue Releases Interleukin-6, but Not Tumor Necrosis Factor-Alpha, in Vivo. J.Clin.Endocrinol.Metab 1997;82(12):4196-200.

45. Fried, S. K., Bunkin, D. A., and Greenberg, A. S. Omental and Subcutaneous Adipose Tissues of Obese Subjects Release Interleukin-6: Depot Difference and Regulation by Glucocorticoid. J.Clin.Endocrinol.Metab 1998;83(3):847-50.

46. Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., and Harris, T. B. Elevated C-Reactive Protein Levels in Overweight and Obese Adults]. JAMA 8-12-1999;282(22):2131-5.

47. Yudkin, J. S., Stehouwer, C. D., Emeis, J. J., and Coppack, S. W. C-Reactive Protein in Healthy Subjects: Associations With Obesity, Insulin Resistance, and Endothelial Dysfunction: a Potential Role for Cytokines Originating From Adipose Tissue? Arterioscler.Thromb.Vasc.Biol. 1999;19(4):972-8.

48. Bastard, J. P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., Laville, M., Vidal, H., and Hainque, B. Elevated Levels of Interleukin 6 Are Reduced in Serum and Subcutaneous Adipose Tissue of Obese Women After Weight Loss. J.Clin.Endocrinol.Metab 2000;85(9):3338-42.